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A new method for extracting earthworms from soil cores, with a comparison of four commonly used methods for estimating earthworm populations

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1. Introduction

Methods of estimating earthworm populations have been reviewed by Bouché (1969) and Satchell (1971). Careful handsorting under a good light was regarded as the most accurate method although, when the sorting was repeated, small specimens were still found. The surface application of 0.5 % formalin solution is known to be less efficient than handsorting with the exception of *Lumbricus terrestris* L. populations but is used where it can be calibrated against handsorting, and where correction factors for temperature and soil moisture are available.

Much of New Zealand's permanent improved pasture is on steeply sloping land, distant from research centres and with limited vehicular access. Improved pastures on flatter land in the North Island tend to be on redeposited, poor draining, heavy soils. These factors increase the difficulties of estimating earthworm populations using conventional methods. In improved pastures in New Zealand the predominant earthworm species are the introduced European Lumbricidae *Allolobophora caliginosa* (Savigny) and *Lumbricus rubellus* Hoffmeister. These species are topsoil worms tending to live within the pasture root-mat during the wetter months from autumn to early summer (April to November). Both species burrow horizontally rather than vertically and do not make permanent tunnels, consequently they are difficult to extract from the soil using vermifuges. Handsorting is laborious, and efficiency depends on the density of the root-mat, the clay content of the soil and, if sorting is done in the field, the weather conditions at the time of sampling.

Because of these factors a method of extracting earthworms from soil is required which:

- is quicker and less laborious than handsorting, allowing more samples to be taken
- is effective on heavy soils which cannot be easily hand sorted when wet
- is independent of the slope of the ground and of the drainage characteristics of the site
- does not involve transport of large volumes of soil, nor leave large holes in the field site
- gives consistently good results even in poor weather conditions
- gives a good recovery of small worms
- gives a good recovery of species from within the root mat.

2. Methods

In the present study four methods of earthworm extraction were tested on the same soil type (Tokomaru silt loam, a heavy soil on flat ground near Palmerston North, New Zealand) on the same date (23 September 1977). The four methods used in this initial comparison were: careful handsorting in the laboratory, surface application of formalin solution in the field (Satchell 1971) heat extraction over water for six hours in an extractor similar to that of Kempson, Lloyd & Ghelardi (1963), and handsorting in the field. Except for the surface applied formalin in which the sampled plots were 0.5 m^2 (with 5 replicates) all the estimates were based on a minimum of 15 soil cores of 16 cm diameter (1/50 m^2) to a depth of 15 cm, in all cases well below the root mat. A 16 cm diameter core was found to be most suitable. A square cut turf 17 m × 17 mm tended to crumble at the corners during extraction and a 10 cm core had a high proportion of damaged worms. The 16 cm diameter circular core gave satisfactory numbers of undamaged worms and was therefore selected as a standard sample unit.

A new method which fulfills most of the criteria listed above combines soil coring and chemical expulsion of worms. At the sample site, soil cores (16 diameter and 15—20 cm deep) were cut and placed turf side uppermost in a 10,000 ml plastic bucket[1]). About 1,200 ml of 0.2 % formalin solution (5 ml of commercial grade 40 % formalin/1,000 ml water) was poured slowly onto the core until the level of the solution was just below the turf surface. After at least one hour of immersion during which the worms moved out of the core, the core was lifted out of the bucket, any worms picked off the surface, and the core replaced in the ground. The formalin solution in the bucket was then poured through a 2 mm sieve and the worms collected into 4 % formalin. The populations estimated by the immersion method were compared with careful handsorting of a further set of soil cores taken at the same time.

The efficiency of different concentrations of irritant immersion solution, both formalin and potassium permanganate, and of 0.2 % formalin at different temperatures were tested. The concentrations of formalin were 0.5 % and 0.2 % and of potassium permanganate were 5 g/litre and 2 g/litre. Four temperature treatments were tested with the 0.2 % formalin only. Cores (15 × 16 cm/treatment) were cut and stored at constant 5 °C, 15 °C or 20 °C for two days before extraction. The fourth set of 15 cores were stored at out-side ambient temperatures (mean min. 3 °C, mean max. 13 °C). The cores were extracted in solutions at the same temperatures as the storage temperatures. The rate at which worms left the cores was also determined by sieving the solution at 30 minute intervals and returning the core to the solution.

In August 1978 the first three methods were compared with a new method on the same soil type. Subsequently the new immersion method was compared with laboratory handsorting on a second soil type (steeply sloping volcanic ash soil Mahoenui steepland soil near Te Kuiti, New Zealand) and with surface application of formalin on a water-logged heavy soil (Kokotau silt loam near Masterton, New Zealand).

3. Results

Handsorting in the laboratory gave the highest estimates of population density. Compared with handsorting the other methods were unsatisfactory but handsorting is too time consuming to be a useful routine methop. Handsorting in the field was both laborious and inefficient and was tested only once.

The population densities estimated by the five different methods are shown in Table 1. In the first series of tests, handsorting in the laboratory and heat extraction gave the highest density estimates, handsorting in the field gave only 42 % of the maximum estimate and surface application of formalin only 47 %. In August 1978 the new formalin immersion method was compared with laboratory handsorting, heat extraction and surface application of formalin on the same soil type. The immersion method and handsorting gave similar high density estimates, surface applied formalin gave the lowest estimate (17 % of the highest estimate) with heat extraction giving an intermediate estimate of density (40 % of the highest estimate). Only two methods were compared on the heavily waterlogged soil at Masterton, and under these conditions surface application of formalin extracted only 23 % of the worms found by the immersion method. When cores from within the formalin sprayed quadrats were hand-sorted in the laboratory, a further 42 % were found bringing the total extracted from these cores to 65 % of the population estimated by the immersion method.

The extraction efficiencies of different immersion solutions and the rates at which worms left the soil in different solutions are shown in Table 2. The 0.2 % formalin treatment was the most effective and extracted worms in good condition. Eighty eight percent of the total number of worms were extracted in the first 30 minutes and 93 % in the first hour. The effects of temperature (Table 3) are probably not significant in the field in most conditions. Even at the cool temperature of 5 °C over 90 % of the worms were extracted in an hour, thus there is no need to correct population estimates for temperature. In areas where ground frost occurs however, it may be necessary to establish a correction factor.

The amount of labour involved in obtaining each estimate of earthworm population has been taken as the time the operator actually spent using the method. The time during which the soil cores were in the heat extractor is not included in the labour estimate as the operator was free to do other work. The working time per sample and the number of samples it is pos-

1) The method was devised for use on soils which cannot easely be sieved or broken up when moist. Soils which require a wire cage to prevent disturbance may possibly be more easely sieved for worms.

Table 1. Earthworm population density estimates (Nos/m² ± SE) for three soil types and five extraction techniques

		Tokomaru silt loam (23. 9. 77)	Tokomaru silt loam (2. 8. 78)	Masterton silt loam (27. 7. 78)	Te Kuiti volcanic ash (21. 8. 78)
Hand sorting in laboratory 20 × 16 cm cores to 15 cm deep	**Total worms**	**772 ± 78.6**	**1,206 ± 162.9**	—	**285 ± 49.5**
	A. caliginosa	525 ± 76.1	809 ± 115.6	—	135 ± 29.4
	L. rubellus	247 ± 51.2	397 ± 84.2	—	150 ± 35.6
Hand sorting in field 20 × 16 cm cores to 15 cm deep	**Total worms**	**329 ± 48.6**	—	—	—
	A. caliginosa	252 ± 31.3	—	—	—
	L. rubellus	77 ± 14.8	—	—	—
Heat extraction 15 × 16 cm cores to 15 cm deep	**Total worms**	**684 ± 54.7**	**521 ± 28.4**	—	—
	A. caliginosa	274 ± 9.6	198 ± 11.2	—	—
	L. rubellus	410 ± 64.8	323 ± 56.4	—	—
Surface applied formalin 0.5 m² quadrats ×5	**Total worms**	**366 ± 29.4**	**216 ± 20.8**	**308 ± 23.1**	—
	A. caliginosa	296 ± 24.6	190 ± 13.8	292 ± 16.1	—
	L. rubellus	70 ± 11.9	26 ± 4.8	16 ± 3.6	—
Formalin immersion 0.2% for 90 mins 15 × 16 cm cores to 15 cm	**Total worms**	—	**1,299 ± 212.0**	**1,360 ± 197.5**	**325 ± 67.7**
	A. caliginosa	—	918 ± 154.7	1,050 ± 162.5	77 ± 12.6
	L. rubellus	—	381 ± 39.8	310 ± 23.6	274 ± 67.4
Hand sorting after surface applied formalin 15 × 16 cm cores to 15 cm	**Total worms**	—	—	**572 ± 42.9**	—
	A. caliginosa	—	—	344 ± 27.1	—
	L. rubellus	—	—	128 ± 21.0	—

Table 2. The mean number and percentage of worms leaving soil cores at 30 minute intervals when immersed in different irritant solutions (15 cores/treatment)

Irritant Solution	30 mins No ± SE	%	60 mins No ± SE	%	90 mins No ± SE	%	**Total**
0.2% formalin	24.0 ± 3.3	*93*	1.4 ± 0.3	*5*	0.6 ± 0.3	*2*	**26.0 ± 4.2**
0.5% formalin	16.4 ± 2.5	*79*	3.2 ± 0.4	*15*	1.3 ± 0.5	*6*	**20.9 ± 2.2**
5 g/l potassium permanganate	10.5 ± 1.2	*75*	3.1 ± 0.2	*22*	0.4 ± 0.4	*3*	**14.0 ± 1.4**
2 g/l potassium permanganate	16.7 ± 2.8	*93*	0.9 ± 1.7	*5*	0.4 ± 0.2	*2*	**18.0 ± 2.7**

Table 3. The number and percentage of worms leaving soil cores immersed in 0.2% formalin at 30 minute intervals after storage at different temperatures (15 cores per treatment)

Storage temperature	30 mins No ± SE	%	60 mins No ± SE	%	90 mins No ± SE	%	**Total**
Ambient	17.1 ± 3.7	*86*	2.5 ± 0.6	*12*	0.3 ± 0.2	*2*	**19.9 ± 4.1**
5 °C	18.2 ± 1.7	*86*	1.9 ± 0.4	*9*	1.0 ± 0.3	*5*	**21.2 ± 1.8**
15 °C	12.3 ± 1.1	*88*	1.2 ± 0.2	*9*	0.5 ± 0.2	*3*	**14.0 ± 1.3**
20 °C	18.3 ± 1.5	*87*	1.9 ± 0.4	*9*	1.0 ± 0.2	*4*	**21.1 ± 1.5**

Table 4. The working time taken per sample unit and the number of units which can be processed by one person in 2 hours

Method	Time per sample unit (mins)	No of units in 2 hours
Hand-sorting in the laboratory	30—45	3—4
Hand-sorting in field	10—20	5—10
Heat extraction	10	12
Surface applied formalin	30	4
Formalin immersion	4—6	20

Table 5. The percentage of each species and of small worms (2 cm) extracted by different methods. Tokomaru silt loam

Method	% *A. caliginosa*		% *L. rubellus*	
	Total	2 cm	Total	2 cm
Hand-sorting laboratory	*67*	*38*	*33*	*47*
Hand-sorting in field	*77*	*12*	*23*	*2*
Heat extraction	*38*	*27*	*62*	*64*
Surface applied formalin	*88*	*10*	*12*	*23*
Formalin immersion	*71*	*39*	*29*	*58*

sible for one operator to process on two hours are shown in Table 4. The most laborious methods were handsorting, both in the laboratory and in the field. As SATCHELL (1971) showed, any reduction of the time spent on each sample unit is likely to reduce the efficiency of the method, and operator fatigue is a significant factor in reducing efficiency when large numbers of cores are taken. Heat extraction was less laborious but may give variable density estimates. Surface application of formalin was almost as time-consuming as handsorting because one

operator cannot usually collect emerging worms from more than two quadrats at once, and worm emergence may not have finished even after 30 minutes. Formalin immersion in the field was most efficient and one person can take over 20 sample units in two hours. When 15 to 20 cores are taken at each field site the time taken to dig the cores and pour on the formalin is such that the first cores are almost ready to be sieved by the time all the cores have been immersed. The time required for sieving and collection into 4% formalin ensures that even the most recently immersed cores will undergo at least one hour's immersion. (At Te Kuiti on steepland pasture soil in adverse weather conditions, two operators extracted worms from 50 cores in two hours).

The percentages of the two species extracted by different techniques and the percentage of the total extracted worms smaller than 2 cm are shown in Table 5. The two species of worms occurring in the pastures were extracted in different proportions by different techniques. Heat extraction gave proportionately more *L. rubellus* (62%) and surface-applied formalin proportionately fewer *L. rubellus* (12%). Handsorting in the laboratory and formalin immersion in the field gave similar proportions of the two species in the populations but by handsorting in the field the proportion of *L. rubellus* was reduced. Some of these differences reflect the known differences in the ecology of these two species. *L. rubellus* tends to occupy the crowns of the plants and the top 1—2 cm of soil whereas *A. caliginosa* is active within the top 1—10 cm of soil. During heat extraction the turf is inverted over water and the surface dwelling *L. rubellus* will have a better chance of escaping than *A. caliginosa* which may get trapped in the drying soil before reaching the lower surface. Small *L. rubellus* are hard to see because their dark colour matches that of ryegrass tillers, so proportionately fewer of this species would be found in hand-sorted samples. The very poor recovery of *L. rubellus* using surface-applied formalin may be due to the difficulty of seeing *L. rubellus* against the turf and the possibility that worms near the soil surface will get sufficient contact with the formalin solution to immobilise them before they can emerge to the surface. Even with very careful handsorting, small worms are missed and those that are collected may be damaged. Heat extraction and formalin immersion gave the most reliable estimates of the numbers of very small worms.

4. Conclusions

The new method for extracting earthworms was both quick and efficient. Under New Zealand conditions, where the bulk of the earthworm population is active within 10 cm of the soil surface, two workers could extract up to 100 cores in the field in a day. Species which live deeper in the soil may still be extracted by watering with formalin or handsorting after the turf mat has been cut away. The method should be calibrated against careful handsorting for each soil type and a correction factor established for very cold conditions.

The major advantages of the new method for top soil species are that a large number of samples can be taken, there is no need to transport soil away from the sample site and the method is not destructive of the sampling area.

5. Acknowledgements

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5. Summary

Four methods for estimating earthworm populations; handsorting in the laboratory, handsorting in the field, heat extraction over water, and surface application of formalin, were found to be unsatisfactory under New Zealand conditions and a new method has been developed. Soil cores (16 cm diameter) are immersed in 0.2% formalin solution (5 ml commercial grade 40% formalin/1,000 ml water) in buckets in the field. Worms move out of the cores and are collected by sieving the formalin solution after one hour. Between 15 and 20 cores can be taken in 2 hours and the method extracts over 90% of the top-soil species (*Allolobophora caliginosa* Sav. and *Lumbricus rubellus* Hoff.) which can be found by careful handsorting. There is no need to transport soil to the laboratory and damage to the field site is minimised.

6. References

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